

Nonreceptor Tyrosine Kinases in Prostate Cancer

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Abstract

BACKGROUND: Carcinoma of the prostate (CaP) is the most commonly diagnosed cancer in men in the United States. Signal transduction molecules such as tyrosine kinases play important roles in CaP. Src, a nonreceptor tyrosine kinase (NRTK) and the first proto-oncogene discovered is shown to participate in processes such as cell proliferation and migration in CaP. Underscoring NRTK's and, specifically, Src's importance in cancer is the recent approval by the US Food and Drug Administration of dasatinib, the first commercial Src inhibitor for clinical use in chronic myelogenous leukemia (CML). In this review we will focus on NRTKs and their roles in the biology of CaP. **MATERIALS AND METHODS:** Publicly available literature from PubMed regarding the topic of members of NRTKs in CaP was searched and reviewed. **RESULTS:** Src, FAK, Jak1/2, and ETK are involved in processes indispensable to the biology of CaP: cell growth, migration, invasion, angiogenesis, and apoptosis. **CONCLUSIONS:** Src emerges as a common signaling and regulatory molecule in multiple biological processes in CaP. Src's relative importance in particular stages of CaP, however, required further definition. Continued investigation of NRTKs will increase our understanding of their biological function and potential role as new therapeutic targets.

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Keywords: Nonreceptor tyrosine kinase, prostate cancer, Src, FAK, ETK.

Introduction

Carcinoma of the prostate (CaP) is the most commonly diagnosed cancer in American men, consisting of more than 33% of all new cancer cases. Though many patients are diagnosed with CaP, it has a relatively low mortality rate when compared to other cancers. Nevertheless, it remains the third leading cause of cancer-related deaths in men in the United States, with about 27,350 estimated CaP-related deaths in 2006 in the United States [1]. Because CaP growth is facilitated by androgen exposure and because androgen withdrawal leads to apoptosis of CaP cells, the current treatment of choice for recurrent or metastatic CaP includes castration through chemical or surgical means. Nearly all patients, however, relapse with androgen-

independent (AI) disease after androgen ablation therapy. Ultimately, the uncontrolled growth of AI metastatic tumors leads to patient mortality.

Tyrosine kinases (TKs) are signaling molecules well known for their roles in human diseases such as diabetes and cancer. Indeed, v-Src sarcoma (Schmidt-Ruppin A-2) viral oncogene homolog (Src), a nonreceptor tyrosine kinase (NRTK), was the first proto-oncogene discovered. More than a quarter of a century has passed since the discovery of Src, and the studies on TKs are coming to fruition with the development and use of tyrosine kinase-based target-specific therapy such as Gleevec, Iressa, and Herceptin for therapy against chronic myelogenous leukemia (CML), lung cancer, and breast cancer, respectively. Dasatinib, a dual Src/v-Abl Abelson murine leukemia viral oncogene homolog (Abl) inhibitor with anti-migratory activity in prostate cancer cells in culture was recently approved by the US Food and Drug Administration for use in patients with CML [2]. Further underscoring the importance of NRTKs, AZD0530 is another dual Src/Abl inhibitor that is currently in multicenter phase II clinical trials for multiple types

Abbreviations: Abl, v-Abl Abelson murine leukemia viral oncogene homolog; AI, androgen-independent; Akt, v-akt murine thymoma viral oncogene homolog 1; AR, androgen receptor; ARG, Abelson-related gene; Bcr, breakpoint cluster region; Brk/PTK6, breast tumor kinase/protein tyrosine kinase 6; BPH, benign prostatic hypertrophy; BRCA1, breast cancer susceptibility gene 1; CaP, carcinoma of the prostate; CML, chronic myelogenous leukemia; CRKII, v-crK avian sarcoma virus CT10 oncogene homolog; CSK, C-terminal Src kinase; DOC-2/DAB2, differentially expressed in ovarian cancer-2/disabled-2; EGF, epidermal growth factor; ER, estrogen receptor; ERK1/2, extracellular signal-regulated kinase 1/2; ET1, endothelin; ETK/BMX, endothelial/epithelial tyrosine kinase/bone marrow X kinase; FAK, focal adhesion kinase; FeR, FpS/FeS-related tyrosine kinase; FeS/FpS, feline sarcoma oncogene/fujinami avian sarcoma viral oncogene homolog; FGR, Gardner-Rasheed feline sarcoma viral (v-FGR) oncogene homolog; Fyn, Fyn oncogene related to Src, FGR, Yes; HIF-1 α , hypoxia-inducible factor 1 α ; IGF-1, insulin-like growth factor 1; IL, interleukin; Jak, Janus kinase; KAI1/CD82, Kangai 1/cluster designation 82; Lck, lymphocyte-specific protein tyrosine kinase; Lyn, v-Yes-1 Yamaguchi sarcoma viral-related oncogene homolog; LPA, lysophosphatidic acid; Met, met proto-oncogene (hepatocyte growth factor receptor); MMP, matrix metalloproteinase; NEP, neutral endopeptidase; NRTK, nonreceptor tyrosine kinase; p130CAS, p130 CRK-associated substrate; PAK1, p21-associated kinase 1; PDGF, platelet-derived growth factor; PI3K, phosphatidylinositol 3-kinase; PKC, protein kinase C; PSA, prostate-specific antigen; PTEN, phosphatase and tensin homolog; PYK2/CAK β , proline-rich tyrosine kinase 2/cell adhesion kinase β ; Raf, v-raf-1 murine leukemia viral oncogene homolog 1; Ras, v-Ha-ras Harvey rat sarcoma viral oncogene homolog; SH, Src homology; Src, v-Src sarcoma (Schmidt-Ruppin A-2) viral oncogene homolog; STAT, signal and transducer of transcription; SYK, spleen tyrosine kinase; Tec, Tec protein kinase; TGF, tumor growth factor; TIMP, tissue inhibitor of metalloproteinase; TKIP, tyrosine kinase inhibitor peptide; TnK, tyrosine kinase nonreceptor; Tyk2, tyrosine kinase 2; VEGF, vascular endothelial growth factor; Yes, v-Yes-1 Yamaguchi sarcoma viral oncogene homolog 1

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of malignancies, including prostate cancer. In this review we will focus on each of the NRTKs and what is known about their respective roles in the biological processes of cell proliferation, migration, invasion, apoptosis, and angiogenesis in CaP.

There are several NRTK families. These are classified based on their structural similarities (Figure 1): Abl, tyrosine

kinase nonreceptor (TnK), C-terminal Src kinase (CSK), focal adhesion kinase (FAK), feline sarcoma oncogene/fujinami avian sarcoma viral oncogene homolog (FeS), Janus kinase (JaK), Src, Tec protein kinase (Tec), and spleen tyrosine kinase (SYK). Though these NRTK families are extensively and individually reviewed elsewhere, this

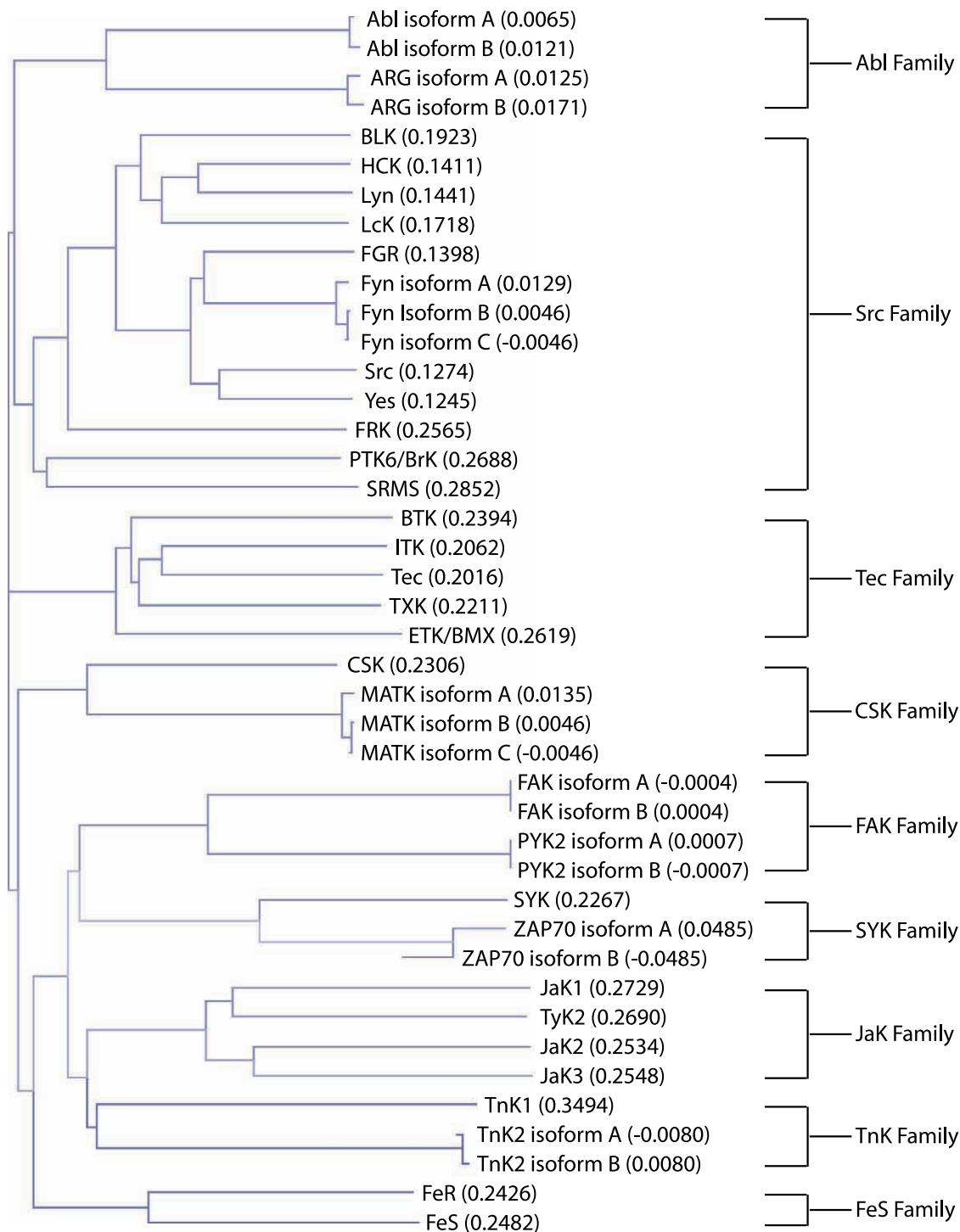


Figure 1. NRTK families and their members in a guide tree. Protein sequences are obtained from Entrez Gene and aligned using Vector NTI Advance software (Invitrogen, Carlsbad, CA). Vector NTI Advance uses the neighbor-joining method of phylogenetic tree construction by Saitou and Nei [127]. The numbers in parentheses after each kinase reflect the calculated distance values between pairs of analyzed sequences.

is the first time they are summarily discussed in relation to CaP.

Profiles of NRTKs in CaP

In 1996, Robinson et al. [3] led the first attempt at profiling the expression of TKs in CaP. Using a modified and improved reverse transcriptase–polymerase chain reaction approach, they identified nine NRTKs expressed in CWR22, a CaP xenograft. NRTKs include lymphocyte-specific protein tyrosine kinase (LcK), v-Yes-1 Yamaguchi sarcoma viral oncogene homolog 1(Yes), Abl, Abelson-related gene (*ARG*), JaK1, tyrosine kinase 2 (TyK2), and endothelial/epithelial tyrosine kinase/bone marrow X kinase (ETK/BMX). Fur-

thermore, *ARG* was found in several other CaP cell lines, which include PC-3, DU145, and LNCaP. In a similar study, Moore et al. [4] used degenerate polymerase chain reaction against conserved kinase catalytic subdomains and found that Abl, JaK1, JaK2, and TyK2 are expressed in surgically removed CaP tissues. In CWR22Rv1, DU145, LNCaP and PC3 cell lines, 18 NRTKs are expressed. This was confirmed by our internal data and also cross-referenced with several published reports (Figure 2).

Src Family

As the first human proto-oncogene discovered, Src’s history spans nearly a century and has been extensively reviewed [5–22]. Members of the Src family include B lymphoid

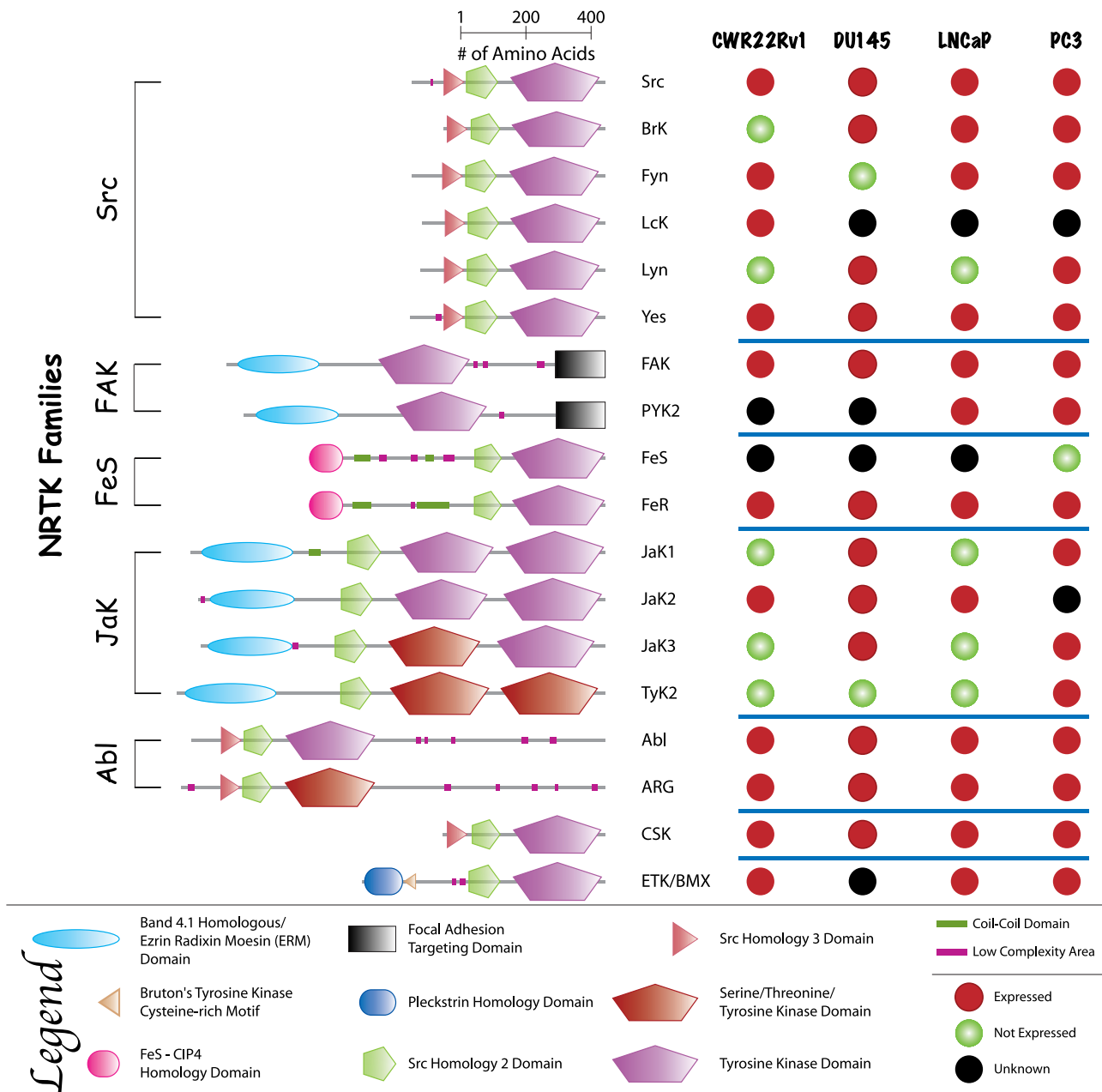


Figure 2. Summary of NRTK mRNA or protein expression in CWR22Rv1, DU145, LNCaP, and PC3 cell lines based on internal data and published reports. NRTK domain drawings and domain information were derived from Simple Modular Architecture Research Tool (SMART, Heidelberg, Germany).

tyrosine kinase (BLK), breast tumor kinase/protein tyrosine kinase 6 (BrK/PTK6), Gardner-Rasheed feline sarcoma viral oncogene homolog (FGR), Fyn oncogene related to Src, FGR, Yes (Fyn), hemopoietic cell kinase (HCK), LcK, v-Yes-1 Yamaguchi sarcoma viral-related oncogene homolog (Lyn), Src, Src-related kinase lacking C-terminal regulatory tyrosine and N-terminal myristoylation sites (SRMS), Yes, and Yes-related kinase (YRK). Of these, FGR, Fyn, LcK, Lyn, Src, and Yes are expressed in either CaP tumor samples or cell lines. Src, FGR, Fyn, LcK, and Lyn in particular have been the most widely studied in CaP.

Src The premier member of its namesake family, Src is extensively studied in cancer biology. Less is known, however, about Src biology in CaP. Though there are no published reports of Src expression or activation levels in clinical CaP specimens, Src is implicated in CaP through its association with factors that correlate positively with the presence or the progression of CaP disease, such as protein kinase C (PKC) ϵ , endothelial-derived gene 1 (*EG-1*), and a truncated form of c-kit [23–25]. As further evidence of Src's possible involvement in CaP, DRS, a negative Src regulator, is down-regulated in CaP tissues and in prostate intraepithelial neoplasia relative to normal and benign prostate hyperplasia (BPH) tissues [26]. Thus, there is circumstantial clinical evidence that Src plays a role in CaP through its interactions with other factors of significance in CaP.

More is known about Src in CaP *in vitro*. Src is expressed in commonly used CaP cell lines CWR22Rv1, DU145, LAPC-4, LNCaP, and PC-3 (Figure 3). At first glance, Src protein expression levels in CaP cell lines do not positively correlate with the aggressiveness, AI state, or the proliferation rates of these cell lines. It is important to note, however, that wild-type cellular Src is not normally constitutively active. Its main role is to transduce signals of upstream activators. In cancer, the upstream signals may be aberrant, thus leading to improper activation of Src and its downstream pathways. One such pathway in CaP is Src activation by neuroendocrine ligands [27].

Neuroendocrine differentiation in CaP is theorized to be in part responsible for the development of AI CaP through the secretion of neuroendocrine ligands. There is evidence that

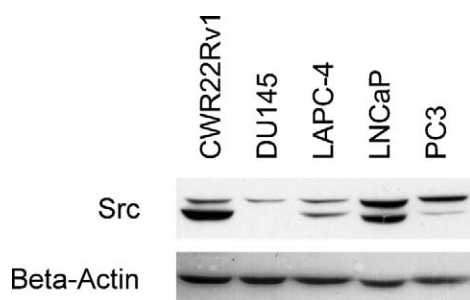


Figure 3. Western blot analysis of total Src protein expression levels in prostate cancer cell lines. Src is shown as a doublet upon probing in most cell lines. Internal overexpression data (not shown) indicate that both bands are Src and that the doublet is not a result of nonspecific probing of other Src family kinase members.

Src takes part in AI cell proliferation. Cyclic adenosine monophosphate (cAMP) analogs are able to activate Src following neuroendocrine differentiation, perhaps secondary to secreted neuroendocrine factors such as gastrin-releasing peptide and lysophosphatidic acid (LPA) [28–31]. LPA is thought to promote cell proliferation through the v-Ha-ras Harvey rat sarcoma viral oncogene homolog (Ras)–v-raf-1 murine leukemia viral oncogene homolog 1 (Raf)–ERK1/2 pathway in Src-dependent fashion. Bombesin, a *Xenopus* gastrin-releasing peptide homolog, can also activate ERK1/2 through Src, possibly through epidermal growth factor (EGF) receptor transactivation [32]. Once ERK1/2 has been activated, it can then activate the androgen receptor (AR) in an AI manner, which promotes cell growth [27,33]. In addition to LPA and bombesin, non-neurotrophic factors such as interleukin-8 (IL-8) and insulin-like growth factor-1 (IGF-1) also promote AI cell growth through Src [34,35].

In addition to cell proliferation, Src also takes part in antiapoptotic pathways in CaP. Bombesin, endothelin (ET1), met proto-oncogene (*Met*), and dihydrotestosterone-activated AR all inhibit apoptosis through Src activation [26,36–38]. There is, however, no consensus mechanism by which Src promotes cell survival. Nuclear factor κ B (NF- κ B)–v-akt murine thymoma viral oncogene homolog 1 (Akt)–p21-associated kinase 1 (PAK1) pathway, MEK1/2–ERK1/2–CREB pathway, and signal and transducer of transcription 3 (STAT3)–dependent down-regulation of B-cell lymphoma leukemia (BCL-xL) and myeloid cell leukemia sequence 1 (MCL-1) are all pathways by which Src inhibits apoptosis [39].

Src is involved in other aspects of CaP biology: cell migration and adhesion. Src interacts with the extracellular signals through the IL-8 receptor, Met, β_1 integrins, Kangai 1/cluster designation 82 (KAI1/CD82), and CD44 [23,34,40,41]. CD44 is a cell surface glycoprotein involved in cell–cell and cell–matrix adhesions. KAI1/CD82 functions as a metastasis suppressor, disrupting integrin-induced Src activation [42]. Intracellularly, Src modulates cell migration and adhesion through its interaction with FAK and p130 CRK-associated substrate (p130CAS) [2].

In addition to cell migration, Src also assists in tumor invasion through its regulation of matrix metalloproteinases (MMPs). MMPs aid in invasion through the degradation of the extracellular matrix. Bombesin promotes Src-dependent tumor progression and metastasis through the activation of MMP9 in conjunction with β_1 integrins [43]. Src inhibition, on the other hand, decreases MMP9 activity levels [2,44].

Induction of angiogenesis by malignant cells is required for continued cell proliferation and metastasis, and vascular endothelial growth factor (VEGF) is a critical angiogenic factor. Src participates in angiogenesis in CaP through the JaK1–STAT3–VEGF pathway [45]. Src activation is also required for VEGF expression in simulated hypoxia environment through increased levels of hypoxia-inducible factor 1 α (HIF-1 α) and activation of STAT3; as additional evidence of Src's involvement in angiogenesis, overexpression of active Src leads to increased VEGF expression [46]. Expression of the melanoma-differentiation–associated gene-7, a Src

inhibitor, on the other hand, inhibits the subsequent downstream STAT3–VEGF pathway [46,47].

Src is also of particular interest in CaP in part because of its interaction with steroid receptors. There is evidence that low amounts of AR and androgen lead to Src activation in the cytoplasm, thereby triggering downstream signaling events independent of AR's transcriptional and DNA-binding activity [38,48]. In fact, dominant negative Src can inhibit DNA synthesis following stimulation with low amounts of synthetic androgen. AR overexpression and higher concentrations of androgen, however, seem to bypass the Src pathway, leading to AR translocation to the nucleus and AR-transcriptional activity–based DNA synthesis.

In addition to the aforementioned activation of Src by androgen-activated AR, Src also associates with AR and estrogen receptor (ER) upon stimulation with estradiol, ultimately resulting in increased cell proliferation [38,49,50]. It is thought that Src serves as a scaffolding protein for the AR–ER complex. Steroidal ligand, however, is not necessary for AR–Src complex formation. Upon EGF stimulation, preformed heterodimers of ER α and AR form a complex with EGF receptor and Src, resulting in the activation and phosphorylation of EGF receptor, DNA synthesis, and cytoskeletal changes [51]. On the other hand, DOC-2/DAB2, a tumor suppressor and a negative Src regulator protein, is reported to inhibit AR's mitotic effects through the disruption of the AR–Src complex [52,53]. Thus, taken together with reports of AI AR activation by Src, AR and Src seem to be able to reciprocally transactivate, depending on the concentration and type of stimulatory ligand.

There are few published reports on cellular elements that negatively regulate Src in CaP. In addition to DOC-2/DAB2, tumor growth factor (TGF) β is reported to decrease both Src expression and its corresponding activity. This is shown by the accumulation of unphosphorylated form of SH2-containing protein (SHC) and a subsequent decrease in complex formation between SHC and growth factor receptor–bound protein 2 (Grb2) [54].

BrK/PTK6 BrK is an Src family member, and little is known about it in CaP. In patient samples, BrK is detected in the nuclei of normal luminal epithelial tissues and well-differentiated tumors, but not in poorly differentiated tumors [55]. Localization of BrK in CaP cell lines LNCaP, which is poorly tumorigenic, and PC-3, which is more aggressive, is primarily nuclear and cytoplasmic, respectively. Though PC-3 expressed more BrK than LNCaP did, BrK is less active in PC-3 cells. Thus, the localization of BrK may play a role in the differentiation of CaP and its aggressiveness.

FGR/Src-2 FGR is an Src kinase family member. It is a negative regulator of phosphatase and tensin homolog (PTEN) and a positive regulator of both Ras and Raf1, thus inhibiting apoptosis and stimulating cell growth, respectively [56]. Though little is known about FGR in CaP, FGR may be overexpressed in CaP, as shown by FGR DNA amplification in patient tumor tissues transitioning from androgen-

dependent to AI states [56]. Thus, FGR may play a role in CaP growth and survival.

Fyn Fyn is an Src family kinase member. It is involved in LNCaP mitogenesis following prolactin stimulation [57]. Though it is suggested that Fyn participates in prolactin-induced cell proliferation through K⁺ ion channels, further studies are necessary in order to elucidate the mechanism of Fyn-modulated prolactin-induced cell proliferation in CaP.

LcK LcK is an Src family kinase member. It is expressed in CWR22 xenograft cells [3]. Little else is known about the role of LcK in CaP.

Lyn Lyn is an Src family kinase member expressed in normal prostate, 95% of primary CaP, and AI PC-3 and DU145 cells [58]. Lyn knockout mice have abnormal prostate gland development. Treatment with KRX-123, a Lyn-specific inhibitor, results in the inhibition of cell growth in DU145 and PC-3 cell lines. DU145 explants in mice treated with KRX-123 were found to also undergo apoptosis. Thus, Lyn seems to play a role in the proliferation and the apoptosis of CaP.

Lyn may also be an important regulator of cell migration in CaP. DU145 cells treated with dasatinib, an Src family kinase inhibitor, have reduced migratory activity [2]. On the other hand, Lyn can bind with neutral endopeptidase (NEP) and act as a competitive inhibitor to the PI3K–FAK complex, resulting in decreased cell migration [59]. Lyn's role in CaP cell migration is therefore inconclusive.

In CaP, Lyn is down-regulated by TGF β and up-regulated by KAI1/CD82 [54,60]. Despite its elevated expression following KAI1/CD82 stimulation, however, Lyn's overall kinase activity was unchanged.

FAK Family

FAK As the predominate member of its namesake family of kinases, FAK is well studied in CaP. Several general reviews of FAK are available [61–71]. Though FAK may play roles in growth, apoptosis, and angiogenesis in CaP, FAK is known primarily for its role in cell motility and cytoskeletal rearrangement, as supported by *in vivo* and *in vitro* evidence. In clinical specimens, FAK expression and activation are uniformly higher in metastatic CaP than in normal and BPH tissues [72,73]. *In vitro* comparison between highly metastatic CaP cell lines and LNCaP, a cell line with lower metastatic potential, shows similar results, with increased expression and activation of FAK in the more aggressive cell lines [74]. FAK's association with molecular mediators of cell migration and adhesions are indicative of its function as well. Activated FAK complexes with β_1 and $\alpha(v)\beta_3$ integrins, molecules involved in cell adhesion [75–78]. As further evidence of FAK's function as a cell motility factor, inhibition of FAK with anti-FAK (pY397) antibody or FAK-related nonkinase (FRNK) resulted in significantly decreased cell migration [79].

Bombesin and IL-8 are both G protein–coupled receptors (GPCR) that activate FAK and stimulate cell migration

[34,79–81]. This is not surprising given FAK's reciprocal transactivation relationship with Src and both IL-8 and bombesin's abilities to activate Src. For bombesin to activate FAK, however, both PKC and an intact cytoskeleton are required [80,82]. Following its activation, FAK then phosphorylates p130CAS, leading to p130CAS–v-crk avian sarcoma virus CT10 oncogene homolog (CRKII) complex formation. Disruption of the p130CAS–CRKII complex by overexpressing KAI1/CD82 results in decreased cell motility [60].

Extracellularly, FAK is activated by integrins, ET1, bombesin, IL-8, and urokinase plasminogen activator (uPA), an invasion and metastasis factor in CaP [83,84]. Intracellularly, it is modulated by Src. It is important to note that Src and FAK activation often go hand in hand. They couple and reciprocally transactivate each other. There are, however, exceptions. FAK activation by autophosphorylation of tyrosine 397 is not Src-dependent; it is adhesion-dependent [74]. On the other hand, phosphorylation of tyrosine 861, which leads to increased FAK activity, is Src-dependent but not adhesion-dependent.

Though FAK is primarily a cell motility regulator, it is also involved in cell proliferation. Similar to cell migration, bombesin-induced FAK-mediated proliferation requires an intact cytoskeleton [80]. A signal downstream of FAK is ETK/BMX, an NRTK critical for bombesin-induced growth [27]. Following FAK activation of ETK/BMX, ETK/BMX subsequently activates AR, thereby inducing cell growth. Interestingly, not only can FAK indirectly activate AR, it can also be activated by membrane-associated AR in a PI3K-dependent manner [85].

In addition to migration and proliferation, FAK may also be involved in CaP angiogenesis and apoptosis. There is evidence that FAK induces VEGF transcription in an ERK1/2-dependent, Rap1-dependent, and Raf-dependent but Ras-independent manner [86]. Increased VEGF transcription may then lead to an increased level of its secreted protein and, thus, angiogenesis. In regard to apoptosis, treatment of cells with proapoptotic factors FTY720 and doxazosin both down-regulate FAK expression for reasons that are not currently known [87,88].

There are few known ways in which FAK is negatively regulated in CaP. Negative FAK regulators include *PTEN*, a tumor suppressor gene with dual phosphatase activity that is frequently deleted in aggressive CaP [89]. FAK may also be indirectly negatively regulated through the formation of the Lyn–PI3K–NEP complex instead of the PI3K–FAK complex [59].

Proline-rich tyrosine kinase 2/cell adhesion kinase β (PYK2/CAK β) PYK2/CAK β is a member of the FAK family of tyrosine kinases. A general review of PYK2 is available [90]. It is expressed in normal prostate epithelia and BPH, but its expression level decreases with increasing grade in CaP [91]. The gene is located on chromosome 8p21.1, a site of frequent deletion in CaP [92].

Though *in vivo* evidence suggests that PYK2 plays a tumor suppressive role in CaP, the *in vitro* evidence of this hypothesis is inconclusive. *In vitro* experiments show that

PYK2 is activated by LPA and tumor necrosis factor α . PYK2 plays a role in the activation of ERK1/2 following LPA stimulation and may thus stimulate cell proliferation [93]. In addition, cells expressing dominant negative PYK2 have decreased proliferation rates. On the other hand, PYK2 indirectly inhibits AR activation through the inactivation of an AR-associated protein, ARA55 [94]. Thus, PYK2's role in CaP may depend on the androgen sensitivity status of the cells in question and requires further investigation and clarification.

FeS Family

The FeS family of NRTKs consists of two members: FeS/FpS and FpS/FeS–related tyrosine kinase (FeR). Little is known about the FeS family in CaP. An examination of CaP cell lines PC-3, PC133, and PC135 failed to detect FeS transcript [95]. FeR expression, on the other hand, was found in CaP cell lines PC-3, DU145, and LNCaP and positively correlated with CaP *versus* normal and BPH tissue samples [96]. Consistent with patient sample data, cells transfected with antisense FeR grew at a slower rate and were unable to grow in an anchorage-independent fashion. In the dog model, a higher FeR expression was found in dividing *versus* resting prostate epithelial cells and in cells displaying basal cell hyperplasia and metaplasia following postcastration estrogen treatment [96]. Thus, FeR is likely a proliferation factor in CaP.

JaK Family

JaK1 The JaK family of kinases is well known for its role in signaling events in cells following cytokine stimulation and its association with the STAT family of kinases. Though JaK1 is present in some clinical CaP specimens, JaK1 is reported to be either negatively regulated or mutated in many CaP cell lines [4,97,98]. LNCaP is found to have both nonsense mutation and repressed JaK1 transcription whereas CWR22Rv1 and LAPC-4 have only nonsense mutations and no known transcriptional repression.

In DU145 cells, which have wild-type JaK1, there are reports that JaK1 associates with breast cancer susceptibility gene 1 (*BRCA1*) [99]. When *BRCA1* is overexpressed, JaK1 and STAT3 become activated. Subsequent inhibition of STAT3 activation results in decreased cell proliferation as well as in apoptosis. Interestingly, inhibition of JaK1 in wild-type DU145 does not result in apoptosis [100]. Thus, it may be possible that although JaK1 activation by *BRCA1* leads to increased JaK1 and STAT3 activation, STAT3 may in fact not be directly downstream of JaK1 in CaP, and their concurrent activation is coincidental.

JaK1 may also play a role in the inhibition of CaP migration and invasion following IL-10 stimulation [101]. Tissue inhibitor of metalloproteinases (TIMP) 1 is an anti-invasion factor. IL-10 is known to activate the JaK1–IL-10E1–TIMP-1 pathway in CaP [102].

JaK2 JaK2 is expressed in some CaP tissues [4]. Similar to JaK1, JaK2 is also activated by *BRCA1* in DU145 cells [99]. It

is interesting to note that although JaK1 inhibition does not result in apoptosis in wild-type DU145 cells, inhibition of JaK2 does [100]. Thus, STAT3 activation in DU145 may be dependent on JaK2 rather than on JaK1. Whether STAT3 is activated by JaK1 or JaK2 in CaP, however, seems to be cell line-dependent [103].

JaK2 may also be involved in cell proliferation in CaP. Tyrosine kinase inhibitor peptide (TKIP) directly inhibits JaK2 autophosphorylation, decreases STAT3 activation, and slows CaP proliferation [104]. Consistent with decreased cell proliferation and STAT3 activation, cyclin D1 level is decreased and cells are arrested in the G₁ phase of the cell cycle following TKIP treatment. Thus, JaK2 may be important for CaP growth through the STAT3 pathway. In addition to STAT3, JaK2 may be of particular importance in CaP through its regulation of STAT5, a factor that positively correlates with the histological grade of CaP [105,106].

TyK2 TyK2 is expressed in some CaP tissues [4]. Though TyK2 may also be involved in CaP migration and invasion and similarly participates in the activation of IL-10E1 following IL-10 stimulation of CaP cells as JaK1, its temporal regulation profile is different from that of JaK1 [101,102].

Members of Other NRTK Families

Abl Abl is well known for its role in the etiology of CML following the formation of the Philadelphia chromosome (t(9:22)) and the breakpoint cluster region (Bcr)–Abl hybrid gene product. Less is known, however, about Abl in CaP. It is known that Abl is expressed in some CaP specimens and that Abl is necessary for retinoblastoma-mediated γ -radiation-induced apoptosis in DU145 cells [4,107]. There is indirect evidence that Abl may be important in CaP. Human spectrin SH domain-binding protein 1 (*Hssh3bp1*) is a gene that binds to Abl, possibly as a negative regulator [108]. A majority (9 of 17) of CaP tumor samples analyzed failed to express *Hssh3bp1*. Furthermore, *Hssh3bp1* is found on chromosome 10p, a region frequently deleted in CaP. Thus, Abl may be circumstantially implicated in CaP through its association with *Hssh3bp1*.

Imatinib mesylate (Gleevec; Novartis, East Hanover, NJ) is a Bcr–Abl inhibitor that is clinically used for the treatment of CML. It also has activity against Kit kinase and platelet-derived growth factor (PDGF) receptor. *In vitro*, Gleevec inhibits CaP cell growth with IC₅₀ in the 10- μ M range [109]. In mice models, however, Gleevec's efficacy against CaP growth is inconclusive with some, but not all, studies showing growth inhibition [110–113].

Similarly, preliminary results from clinical studies also paint a mixed picture. A phase I clinical trial of Gleevec in combination with docetaxel in AI CaP showed a prostate-specific antigen (PSA) decline in 14 of 21 patients, although it is unknown whether the decline can be attributed to Gleevec or docetaxel [114]. In another AI CaP study, Gleevec in combination with zoledronic acid (Zometa, Novartis) showed no clinical effect in 15 CaP patients [115]. Lastly, as monotherapy in 16 patients with androgen-sensitive CaP, Gleevec treatment resulted in nine patients with stable PSA levels

and seven patients with PSA progression [116]. Thus, clinical use of Gleevec as monotherapy in CaP may be ineffective. The efficacy of using Gleevec as an adjuvant therapy to other treatment modalities is presently unknown.

CSK CSK is a well known negative Src regulator [117]. Little is directly known about CSK in CaP other than that it complexes with FAK in metastatic tumors and PC-3 cells [73].

ETK/BMX Discovered in 1994, ETK/BMX belongs to the Tec family of NRTK [118]. In CaP, ETK is downstream of PI3K in the induction of the neuroendocrine differentiation of LNCaP cells following IL-6 stimulation [119]. It is also reported to function as an antiapoptotic factor. Overexpression of ETK confers resistance to apoptosis in CaP cells through its interaction with PI3K [120]. PI3K is not, however, required for ETK activation [27]. Another mechanism by which ETK may protect against apoptosis is through its interaction with p53 [121]. Interestingly, ETK also participates in the apoptotic cascade in CaP cells. Introduction of ETK's C-terminal fragment into PC-3 cells can lead to apoptosis following proteolytic cleavage of ETK by caspases [122].

ETK is also critical for cell proliferation following bombesin stimulation and AR activation in CaP [27]. ETK serves as a signal transducer between Src and FAK upstream and AR downstream. ETK alone, however, is insufficient for AR activation. ETK must be able to reciprocally transactivate with Pim1 before AR activation [123,124].

Other NRTKS SYK and TNK1 are other NRTKs that have been studied in CaP. Virtually nothing is known about their properties and functions in prostate cancer except that the promoter region of SYK is hypermethylated and TNK1 transcript is found in prostate tissues [125,126]. SYK expression may thus be down-regulated in CaP, whereas TNK1 protein expression level remains to be investigated.

Conclusion

Much is known regarding specific NRTKs in CaP (Src, FAK, JaK1/2, and ETK), whereas less is known about the other NRTKs. Perhaps it is not a coincidence that the well-studied Src, FAK, JaK1/2, and ETK kinases are involved in processes indispensable to the pathology of CaP: cell growth, migration, invasion, angiogenesis, and apoptosis. It is therefore imperative that we learn more about these NRTKs through future studies. Although Src, FAK, JaK1/2, and ETK are important in CaP biology, we should not neglect the other NRTKs that may also play important roles in CaP and should also investigate the lesser known NRTKs.

Looking at the current literature of NRTKs in CaP, there emerges a picture of Src being an ubiquitous player in multiple biological processes interacting with numerous players in multiple signaling pathways. Src transduces signals from upstream receptors such as IL-8, EGF, IGF-1, neurotensin, ET1, and HGF/SF to downstream molecules such as FAK, ETK, JAK1/2, STAT3, Ras, ERK1/2, Akt, HIF-1 α , and, of particular significance in CaP biology, AR (Figure 4). Given

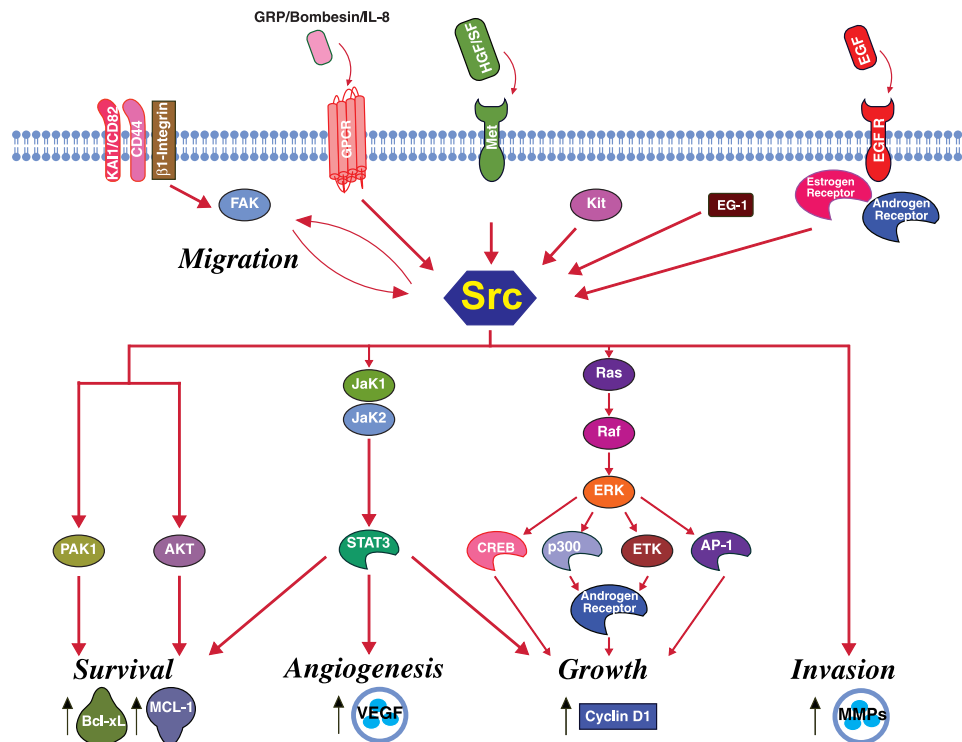


Figure 4. Known Src pathways in prostate cancer. The close proximity of molecules not connected with arrows denote physical association. Red arrows denote activation. Black arrows denote change in levels of molecule. Figure templates were provided by BioCarta (San Diego, CA).

the preponderance of evidence in multiple biological processes linking Src to CaP, Src is likely an important point of pathway convergence in CaP. Perhaps it is not surprising then that Src is currently the only NRTK target in clinical trials for CaP, whereas no NRTK-specific therapy is available for general clinical use in CaP. What remains unclear, however, is Src's relative importance in particular stages of CaP: oncogenesis, growth, survival, AI growth, angiogenesis, and metastasis. Nevertheless, with cancer treatments moving toward targeting specific pathways, it is important that we continue investigating signaling pathways so that we can develop novel therapies through continued research.

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